(such as bacille Calmette-Guérin and adenovirus), and novel recombinant DNA constructs may yet lead to promising efficacy results as a basis for large-scale testing. In the meantime, high-risk uninfected cohorts are now being recruited and the infrastructure put in place in the United States and abroad for efficacy tests of one or more of the most promising candidate HIV-1 vaccines that may become available in several years. The first vaccines used may be only partially effective, but their early introduction might still prevent many infections, reduce viral transmission, delay disease, and provide valuable information that could facilitate future vaccine development. Multiple vaccines that are tailor-made for the predominant HIV strain(s) circulating at different global sites may need to be tested simultaneously. A long-term commitment has been made to vaccine development; this must include realistic expectations and both preclinical and clinical trials.

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Laboratory Evaluation of Inherited Thrombotic Disorders

RECENT ADVANCES IN THE diagnosis of and therapy for thrombotic disease have focused attention on the consulting role of clinical pathologists in the laboratory evaluation of coagulation disorders. Patients referred for evaluation usually have venous thromboembolism, but some may have arterial thrombotic events. Inherited disorders that can be tested for in a coagulation laboratory may result from abnormalities in inhibitors of activated coagulation factors (antithrombin III, protein C, protein S), impaired clot lysis (dysfibrinogenemia, plasminogen deficiency, tissue-plasminogen activator deficiency, excess plasminogen activator inhibitor), or a metabolic disorder associated with vascular disease and thrombosis (homocystinuria).

Although only about 30% of patients with recurrent thrombosis have an inherited disorder identified, the yield of this evaluation can be improved by restricting it to young patients with recurrent thrombosis or patients with thrombosis and a positive family history. The laboratory evaluation should be deferred until two to three months after the acute event (to avoid acute-phase changes that may obscure a correct diagnosis) and after the patient has discontinued anticoagulant therapy. Many laboratories offer both functional and immunologic assays for evaluating these disorders. Because thrombotic disease may result from either quantitative deficiency or a qualitative abnormality of these proteins (for example, antithrombin III or protein C) and immunologic assays may yield normal results in patients with dysfunctional proteins, it is preferable to do functional assays that detect both types of disorders. Appropriate specimen collection, timing, and processing are critical, especially for fibrinolytic assays (tissue-plasminogen activator, plasminogen activator inhibitor). Deficiencies of proteins C and S and antithrombin III are thought to be the most common causes of inherited thrombosis. Previous studies indicating that fibrinolytic abnormalities were common causes of recurrent thrombosis have been recently challenged. A metabolic disorder, heterozygous homocystinuria, has been increasingly associated with arterial vascular disease and should be considered in middle-aged patients with premature arterial thrombosis.

One approach in the laboratory investigation of recurrent thrombosis is to first exclude acquired causes of hypercoagulability—hyperlipidemia, lupus anticoagulant, or malignancy. The presence of venous thrombosis should be initially evaluated with a functional protein C assay and a free protein S antigen assay. If the results of these tests are normal, a functional assay for antithrombin III can be done, followed by testing for dysfibrinogenemia. If the test results are normal, the fibrinolytic system could be evaluated.

Unexplained arterial thrombosis in young patients can be evaluated with an assay of plasminogen activator inhibitor activity, whereas middle-aged patients with premature arterial thrombotic events should be tested for heterozygous homocystinuria using a methionine loading test.

The importance of laboratory monitoring of anticoagulation has been emphasized by studies showing the clinical benefit of adequate heparin and warfarin therapy in treating thromboembolism. Because the responsiveness of commercial laboratory-activated partial thromboplastin time reagents may vary substantially, each laboratory should establish its therapeutic heparin range (activated partial thromboplastin time ratio, 1.5 to 2.5), so that it is equivalent to a heparin level of 200 to 400 U per liter (0.2 to 0.4 U per ml) by protamine titration. Despite the large body of published evidence indicating the clinical importance of using the international normalized ratio format in reporting laboratory prothrombin times, many coagulation laboratories do not use this format, leading to inappropriate anticoagulant therapy for many patients. Increased educational efforts will be necessary to inform practitioners and pathology personnel of the importance of rigorous laboratory control of anticoagulation.

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Enhancing Antibody and Its Role in Acquired Immunodeficiency Syndrome

ANTIBODIES AGAINST VIRAL proteins have traditionally been considered beneficial to the host by preventing infection. This principle has been the mainstay of modern vaccine development. It is now known, however, that certain antibody responses may not benefit the host but may, indeed, benefit the virus. One such phenomenon, antibody-dependent enhancement, increases the infectivity of a variety of viruses from several different families. Antibody-dependent enhancement can occur when nonneutralizing antibodies bind to viral surface proteins. The Fc portion of this antibody can then be bound by Fc receptors on macrophages. Viruses that are resistant to lysosomal degradation can then infect macrophages by this route. A second mechanism has been described whereby nonneutralizing antibodies bind to viral

surface proteins and activate the complement cascade. Complement proteins, specifically C3, are fixed to the virus. This opsonized virus can then bind to complement receptors on macrophages, lymphocytes, or follicular dendritic cells. The virus can thereby infect these cells through this pathway.

Although in vitro antibody-dependent enhancement has been reported for a number of viruses, it has been difficult to show enhanced infections in vivo. The best documented example is dengue virus where clinically substantial dengue hemorrhagic fever and dengue shock syndrome occur in the presence of enhancing antibodies. Indeed, the severity of disease was correlated to the levels of enhancing antibodies. In vitro and in vivo antibody-dependent enhancement has been described for a number of animal viruses as well and is an important concern in veterinary vaccine development. This phenomenon of antibody-dependent enhancement and the viruses for which it has been observed were recently reviewed.

Infection by the human immunodeficiency virus (HIV) is accompanied by potent host immune responses against the virus. Early in HIV infection, patients have measurable neutralizing antibody titers, antibodies that can lyse HIV-infected cells, and cytotoxic lymphocytes that can kill virus-containing $CD4^+$ lymphocytes. In theory, all of these responses should combine to eradicate HIV infection in the host. Nevertheless, despite these immune responses, persons infected by HIV continue to progress through the full gamut of HIV-induced disease and finally die of complications of the acquired immunodeficiency syndrome (AIDS). This fact led investigators to search for possible adverse immune responses that may complicate the host response to HIV infection. In 1987, investigators first reported antibody-dependent enhancement of HIV infection in vitro. Later reports indicated that this enhancement could occur through both the complement and Fc receptor-mediated mechanisms. It has been difficult, however, to show a clear clinical role for enhancing antibodies in HIV infection. The best data on the role of enhancing antibodies in HIV-induced disease may come from studies using animals. Two separate studies using rhesus macaques and the simian immunodeficiency virus (SIV) have shown that enhancing antibody levels increase throughout the disease course and peak before death of the animals from AIDS. This animal model is arguably the best one for HIV infection. It has also been shown that chimpanzees infected with HIV have antibodies that can enhance HIV infection in vitro. Nevertheless, these antibody levels appear to decline the longer the chimpanzee is infected. Chimpanzees do not go on to have AIDS, nor do they become clinically ill following HIV inoculation.

Formidable difficulties are involved in studying enhancing antibodies in a disease that takes years to become clinically apparent. The average length of time for patients to progress to AIDS is 12 years. Even if high levels of enhancing antibodies resulted in a change in disease progression from 12 years to 8 years, it would require a huge clinical sample.

The greatest concerns to practitioners involve the theoretic risk of antibody-dependent enhancement in HIV infection. To date, no one has yet proved that in vivo enhancing antibodies of HIV infection do or do not play a role in disease progression. Circumstantial evidence such as disease progression despite potent immune responses to HIV and vaccine failure in animals suggests that in vivo antibody-dependent enhancement may be a reality. Can the theoretic risks be ignored, thus placing thousands of lives at risk if mass inoculation is given to persons in HIV-endemic areas with vaccines that produce antibody-dependent enhancement?

Despite the protestations of some investigators, mass immunization of many persons in high-risk areas of the world with vaccines that have proved ineffective in animals may occur. If these immunized patients show an increased susceptibility to HIV infection, then enhancing antibodies are important. If such studies progress as planned, we can only hope that antibody-dependent enhancement of HIV infection, unlike dengue virus, will be only an in vitro artifact. At this time, possible risks outweigh any demonstrated benefits to the putative AIDS vaccine being tested. A quick cure is not in sight for this pandemic.

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Iron Deficiency

STUDIES HAVE ASSOCIATED iron deficiency with epithelial changes, impaired immune response, abnormal temperature regulation, deranged catecholamine metabolism, and abnormal physical activity independent of any anemic effects. More recent studies have shown behavioral changes, reduced exercise tolerance, decreased levels of the neurotransmitter γ-aminobutyric acid in brain, altered lipid metabolism, and abnormal myelination of the central nervous system. Many of these diverse effects are reversible with iron therapy, whereas others may be partially reversible or permanent.

Of particular interest has been the effect of iron deficiency on the central nervous system independent of any concomitant anemia. Some parts of the brain contain considerable quantities of iron; iron is required for the activity of enzymes such as hydroxylases and monoamine oxidases that are important in brain function. Iron also influences myelination by its effects on lipid metabolism, causing changes in the fatty acid composition and content of lipids in both peripheral circulation and brain. Brain sphingolipid of iron-deficient animals has shown a pronounced reduction of fatty monoenoic acids such as ω9,C18:1 and ω9,C24:1. Iron supplementation of deficient animals results in at least a partial correction of these deficits. Iron is an essential requirement for desaturase enzyme function in liver, and iron deprivation might be expected to depress comparable desaturase activity in the fetal and neonatal brain. Observations to date show that iron deficiency is associated with dramatic changes in the fatty acid composition of myelin-specific lipids such as cerebroside.

Extensive studies done in children and adults indicate disturbances of behavior and cognitive factors with iron deficiency alone. In one study series, infants having iron deficiency with and without anemia tended to score lower in the Bayley scale of mental development than those without sideropenia. A clear sensory disturbance of taste has been well documented in adults with iron deficiency with and without